REMARKS

Claims 68, 77-78, 80-87, 89-94, 96, 102, 104-105, 125-135, and 137-139 are pending in the application. Claims 87 and 89 were withdrawn from consideration. Claims 68, 77, 78, 80-86, 90-94, 96, 102, 104, 105, 125-135 and 137-139 are under examination. Claims 68, 91 and 130 have been cancelled and Claims 90, 125, 126, 137 and 139 have been amended.

The amendments have been made to place the claims in better form for examination and to further obviate the 35 U.S.C. 102, 103 and 112 rejections as set forth in the Office Action dated September 25, 2008. It is believed that none of these amendments constitute new matter. It is submitted that these amendments obviate the rejections. Withdrawal of these rejections is respectfully requested.

Previous Claim Rejections Under 35 U.S.C. § 102(e) and 103(a):-Withdrawn

The previous rejection of Claims 67-68, 77, 80-84, 126-129 and 137 under 35 U.S.C. § 102(e), on the basis that the claims are anticipated by Lauffer, et al., (US Publication No. 2001-0053539) has been withdrawn by the Examiner in view of Applicant's amendment of claims (dated May 14, 2008).

The previous rejection of Claims 90-94, 96, 104, 130-136 under 35 U.S.C. § 103(a), on the basis that the claims are unpatentable over Lauffer, et al., (US Publication No. 2001-0053539) in view of Mapelli, et al. (U.S. Patent No. 5,519,115). has been withdrawn by the Examiner in view of Applicant's amendment of claims (dated May 14, 2008).

The previous rejection of Claims 133-135 under 35 U.S.C. § 103(a), on the basis that the claims are unpatentable over Lauffer, et al., (US Publication No. 2001-0053539) in view of Mapelli, et al. (U.S. Patent No. 5,519,115) and further in view of Qiu et al. has been withdrawn by the Examiner in view of Applicant's amendment of claims (dated May 14, 2008).

The previous rejection of Claims 78, 86, 102 and 105 under 35 U.S.C. § 103(a), on the basis that the claims are unpatentable over Lauffer, et al., (US Publication No. 2001-0053539) in

in view of Sytkowski (U.S. Patent No. 5,580,853) has been withdrawn by the Examiner in view of Applicant's amendment of claims (dated May 14, 2008).

Rejection Under 35 U.S.C. § 112, Second Paragraph

The Examiner has rejected to Claims 126 and 137 under 35 U.S.C. § 112, second paragraph for being indefinite. The rejection reasons that the phrase "a natural erythropoietin amino acid sequence and the natural immunoglobulin domain amino acid sequence" is unclear because without a sequence or specifying the species of the amino acid sequence (human, mouse, etc.), the metes and bounds of the claim allegedly cannot be determined.

Claims 126 and 137 have been amended to recite that the erythropoietin and immunoglobulin domain amino acid sequences are human sequences.

In view of this amendment, withdrawal of the rejection of Claims 126 and 137 under 35 U.S.C. § 112, second paragraph is respectfully requested.

Rejection Under 35 U.S.C. § 102(e)-Strom et al.:

The Examiner has rejected Claims 68, 77, 78, 80-86, 125-129 and 137 under 35 U.S.C. § 102(e) as being anticipated by Strom, et al. (US Patent No. 6,165,476, filed 10 July 1997). The rejection asserts that Strom, et al., teaches an erythropoietin (EPO) fusion protein wherein an EPO protein is attached to an amino acid sequence comprising an immunoglobulin hinge region sequence (direct fusion between EPO and an Ig domain). The rejection contends that Strom, et al. teaches that the amino acid sequence of the EPO protein can be wild-type human EPO and that the amino acid sequences of the hinge regions include human Ig sequences. In addition, the Examiner asserts that Strom, et al teaches pharmaceutical compositions comprising the fusion protein, making the fusion protein using recombinant technology, purifying the fusion protein using chromatography and that the fusion protein can be in a dimeric form. The Examiner does acknowledge that Strom, et al. does not expressly teach an EC_{50} value as recited in the claims, although the Examiner states that this activity is inherent to the protein since it has the same

structure as claimed. Applicant has amended Claim 125 from which Claims 77, 78, 80-86 and 126-129 depend from to be limited to a full-length Ig domain of IgG-Fc, IgG-C_H and IgG-C_L. In addition, Claim 137 has been amended in a similar manner. Claim 68 has been cancelled.

Additionally, Applicant contends that Strom, et al. describes an Epo fusion protein in which an Epo homodimer is constructed using an Ig hinge region to separate the two Epo proteins. Therefore, the fusion protein of Strom, et al. has the structure Epo - Ig hinge – Epo, which is different than the structure of the claimed fusion protein. Strom's fusion protein contains only the Ig hinge region and not the other parts of the IgG molecule. Strom, et al. does not teach or suggest the idea of joining Epo to an IgG-Fc, IgG-CH or IgG-CL domain to make an Epo-IgG-Fc, Epo-IgG-CH or Epo-CL fusion protein. In column 5, lines 38-52 Strom , et al. states that the fusion proteins are constructed so that the C-terminus of one protein (e.g., Epo) is joined to the N-terminus of the IgG hinge region and the N-terminus of the second protein (Epo) is joined to the C-terminus of the Hinge region to create the Epo-Hinge-Epo homodimer fusion protein. The Ig Hinge region was chosen because it is small and flexible (column 4, lines 16-17).

In contrast, the current amended claim set claims an Epo joined to an IgG-Fc (Hinge-CH2-CH3 domains), and Epo-C_H fusion (CH1-Hinge-CH2-CH3 domains) or C_L domain. Therefore, the Applicant asserts that the EPO fusion of the claimed invention is not anticipated by the EPO fusion protein described by Strom, et al. The EPO fusion protein of the present invention is joined to a much larger Ig region that contains both flexible (Hinge) and non-flexible (CH1, CH2, CH3 domains) regions compared to the EPO fusion protein of Strom, et al. In addition, the EPO fusion protein of the claimed invention contains only a single Epo joined to the Ig domains, which is not the same as the homodimeric proteins taught by Strom, et al.

In addition, the Applicant contends that the method of making the EPO fusion protein as taught by Strom, et al. would not produce the same EPO fusion protein as currently claimed. Strom, et al. used PCR amplification methods to make a direct fusion of the C-terminus of the first Epo protein (referred to as Epo A) to the N-terminus of the Ig hinge domain (oligos for doing this are described in column 14, lines 16-30 of Strom, et al.). The PCR amplified Epo A-

Hinge protein was constructed so that it had a Not I restriction site at the 5' end preceding the Epo coding sequence and a Bam HI restriction site following the end of the Hinge sequence. However, in column 14 lines 6-15 Strom, et al. states that a different method was used to join the C-terminus of the Hinge domain to the N-terminus of the second Epo protein (referred to as Epo B). For this different method, Strom, et al. used PCR amplification methods to change the DNA sequence of Epo B so that the first amino acid of Epo B protein is changed from Ala to Asp, which creates a new Bam HI site at the beginning of the DNA sequence encoding Epo B (oligos are described in column 14, lines 28-30 of Strom, et al.). In the 3' non-coding region of the DNA encoding Epo B, Strom, et al. added an XbaI restriction site (does not change the amino acid sequence of Epo B at this end of the protein). Strom, et al. then cloned the Epo A-Hinge DNA into one plasmid and DNA encoding Epo B protein into a second plasmid. Eventually Strom, et al. cut the plasmid DNA encoding Epo A-Hinge with Not I and Bam HI and isolated the DNA fragment encoding Epo A-Hinge. Strom, et al. then cut out the DNA encoding Epo B using Bam HI and XbaI and isolated DNA encoding Epo B protein. The fragments were mixed together and cloned into a new plasmid that had been digested with NotI and XbaI. Thus, the Bam HI site was used to join the C-terminus of the EpoA-Hinge domain to Epo B. Joining the two DNA fragments is described in column 15, lines 12-28 of Strom, et al. Therefore, Strom, et al. teaches using a Bam HI site to join the C-terminus of the Hinge to the second protein (Epo B). However, if one used a Bam HI site to join the Hinge to other domains as claimed (IgG CH2-CH3 for example), then the amino acid sequence at the beginning of the CH2 domain would need to be changed to accomplish this, just as Strom, et al. had to do with Epo. The amino acids at the beginning of the CH2 domain cannot be encoded by DNA that comprises a Bam HI site. One would have to change the amino acid sequence of the CH2 domain and thus would not have a native full-length sequence of human IgG-Fc domain or IgG-CH or CL. Therefore, Applicant contends that the Ig region taught by Strom, et al. (amino acid sequence comprising the hinge region sequence) does not meet the limitation recited in the amended claims.

In addition, Applicant submits that the DNA sequence given for the IgG1 hinge region in

column 13, lines 60-62 of Strom, et al. does not correspond to a human IgG1 hinge region sequence or sequence of any known human IgG Hinge region. The DNA sequence encodes an additional proline that is not present in the human IgG1 hinge region. The encoded amino acid sequence therefore is Glu-Pro-Lys-Ser-Cys-Asp-Lys-Thr-His-Thr-Cys-Pro-Pro-***Pro***-Cys-Pro (the italicized Pro is the extra one not present in native sequence). Thus, Strom, et al. does not use a native sequence human Ig hinge region as is claimed in the present invention.

Lastly, the Examiner states that the EC50 values are inherent to the fusion protein of the claimed invention and the fusion protein of Strom, et al. because they will have the same structure. Applicant asserts that the fusion protein as now claimed does not have the same structure of the fusion protein as taught by Strom, et al and therefore will not inherently have the same EC50 value.

In view of the foregoing remarks, the fusion protein of the claimed invention is not anticipated by Strom. et al., and withdrawal of the rejection of Claims 68, 77, 78, 80-86, 125-129 and 137 under 35 U.S.C. § 102(e) is respectfully requested.

Rejection Under 35 U.S.C. § 103-Blumberg, et al. in view of Mapelli, et al.:

The Examiner has rejected Claims 90-94, 96, 102, 104, 105, 130-132 and 139 under 35 U.S.C. § 103(a), on the basis that these claims are unpatentable over Blumberg, et al. (U.S. Patent No. 6,485,726), in view of Mapelli, et al. (U.S. Patent No. 5,519,115). The Examiner has stated that Blumberg, et al. teaches conjugating an EPO to an Fc fragment of the constant region of an immunoglobulin, such that the fusion protein can bind to the FcRn receptor and be transported across the epithelial barrier by FcRn mediated-transport. Blumberg, et al. also teaches an example of EPO-Fc fusion, in which the entire mouse EPO sequence was linked at its C-terminus to the Fc fragment through an Ala-Ala-Ala linker. Blumberg, et al also teaches that the DNA encoding EPO may be replaced with DNA encoding any of the other proteins, including human EPO taught in U.S. Patent No. 4,703,008, by using standard techniques to create an Fc fusion protein. Additionally, the Examiner asserts that Blumberg, et al. teaches

recombinant expression and purification of the fusion protein and that the EPO-Fc fusion is in a dimeric form. The Examiner does point out that Blumberg, et al. does not teach a peptide linker that consists of a mixture of 2, 4 or 7 amino acid residues (consisting of glycine and serine) wherein the peptide linker is SEQ ID NO:1 of the current application, however the Examiner states that it would be obvious to one of ordinary skill in the art to combine Blumberg, et al. with the teachings of Mapelli, et al. (teaches use of a small bridge-5 amino acids or less, in the construction of oligopeptides) to produce the resulting fusion protein of the present invention.

Applicant respectfully disagrees with the Examiner's above statements however; in the interest of expediting prosecution Claim 90 has been amended to full-length human Ig domains and a peptide linker that consists of glycine and serine. Claim 91 has also been canceled.

Applicants submit that Blumberg, et al., U.S. Patent No. 6,485,726, is not effective prior art against the invention as claimed in the rejected claims, because the claimed subject matter was invented by the present inventors prior to the effective date of the the material referenced by the Examiner in columns 12 and 13 regarding construction of the EPO-Fc fusion. This material was added in Pat. No. 6,485,726 and does not appear in his priority document of Pat. No. 6,030,613. The effective prior art date for this new disclosure is the filing date of Pat. No. 6,485,726 or July 24, 1998. Enclosed herewith is a Declaration under 37 CFR § 1.131. This Declaration has been executed by one of the inventors, George Cox. A copy of the Declaration signed by the other inventor, Daniel Doherty will be submitted supplementary. This Declaration provides evidence of conception of the invention as claimed in these claims at a date prior to July 24, 1998, followed by diligence beginning from a date prior to July 24, 1998, to the constructive reduction to practice of the invention as claimed in the presently rejected claims. As required by 37 CFR § 1.131, the Declaration affirms that the acts relied upon to establish actual reduction to practice were carried out in the United States. Therefore, Applicants submit that Pat. No. 6,485,726 is not an effective reference against the presently rejected claims, and the rejection should be withdrawn on this basis.

In addition, even if, *arguendo*, Pat. No. 6,485,726 was an effective reference, Applicant contends that Blumberg is, et al., does not actually teach or suggest an EPO-IgG fusion protein as claimed, as asserted by the Examiner, as explained in detail below.

The only time Blumberg, et al. mentions an Epo-IgG fusion protein is in a hypothetical discussion that begins at the bottom of column 12, lines 63-67 and continuing onto column 13. Blumberg, et al. does not describe construction of an Epo fusion protein in any of the detailed Examples. Thus it is not clear that Blumberg, et al. ever made the fusion protein or that the fusion protein was biologically active. In column 12, lines 62 onward, Blumberg, et al. states the conjugate consists of an Fc fragment (starting with amino acids DKTH) at the N-terminus of the CH1 domain, … and continuing to the SGPK sequence at the end of the CH3 region. The Applicant asserts that the DKTH sequence Blumberg, et al. mentions is actually in the Hinge region of the IgG domain, and not the CH1 domain. The entire CH1 domain would be deleted if one starts with DKTH. Further, it is known in the art that the Hinge region of IgG1 begins with the sequence EPKSCDKTH… . Blumberg, et al. begins at the DKTH sequence because they want to use a Sal I site that exists in their plasmid preceding the D of the DKTH sequence. Thus the Applicant asserts that the construct of Blumberg,e t al. will not contain the entire full-length human IgG-Fc hinge region sequence – it will be missing the first EPKSC amino acids. It is known in the art that the last C in the missing amino acids is important for forming a disulfide bridge between two IgG domains; thus by removing the C, interference with dimerization of the fusion proteins will occur. Figure 1A of Blumberg, et al. provides the DNA and amino acid sequence of their IgG domain beginning at the SalI site (it appears they have cloned only a portion of the IgG-Fc region beginning at DKTH into a vector. The VV amino acids preceding the DKTH sequence come from their plasmid and are not present in IgG sequences). Blumberg, et al. presented all 3 possible reading frames of the DNA – the correct reading frame is the 3rd reading frame, beginning VVDKTHTCPP … Sal I recognizes the DNA sequence GTCGAC and cuts in between the GT. This sequence precedes the DNA encoding the DKTH amino acid sequence. Blumberg, et al. in column 13, lines 12-27 states that the Epo gene will be modified at

its C-terminus to add 3 alanine residues followed by a SalI restriction site. Alanine is encoded by GCX, where X can be A, C, T or G. Therefore, the Epo sequence of Blumberg, et al would have to be encoded by Epo - GCX GCX GCX GTC GAC. The final GAC encodes the D of the DKTH sequence. GTC encodes valine. Therefore, Blumberg, et al's Epo construct would be separated from the Fc domain by at least 4 amino acids (Ala Ala Ala Val), and not 3 as the Examiner has stated. Blumberg, et al. does not teach or suggest a human EPO fusion protein linked to a full-length human immunoglobin domain via a peptide linker. Therefore, it would not be obvious to use the teachings of Blumberg, et al. to produce the fusion protein of the claimed invention.

In regards to Mapelli, et al. the Applicant maintains that this reference is not at all directed to the production of fusion proteins in general, let alone an EPO-Ig fusion protein, which is a fusion of two large proteins with very different structures. EPO alone contains about 166 amino acids, which is much larger than the shorter peptides used in the monomers of Mapelli, et al. The EPO-Ig fusion proteins are far more complex than the simple oligomers contemplated by Mapelli, et al, because EPO-Ig fusion proteins also interact to form disulfide-linked dimers, which can negatively affect their bioactivity. Thus, EPO and Ig proteins can not properly be considered to be peptide monomers as used by Mapelli, et al. Mapelli, et al., does not teach or suggest the use of full length proteins and requires that the bridge promote inter-monomer interactions. Mapelli, et al. teaches that this is desirable so that the oligopeptides can be transported across (plant) cell membranes (col. 23, lines 23-30) and provide the ability to protect against plant pathogens which is related to the ability to form aggregates (col. 23, lines 35-44). These concerns are not relevant in the production of EPO-Ig fusion proteins (which are not oligopeptides as defined by Mapelli, et al.) and thus there is no reason to look to Mapelli, et al., for guidance on a form of peptide linker in an EPO-Ig Fusion. Accordingly, Mapelli, et al. does not teach or suggest the presently claimed invention, either alone or in combination with Blumberg, et al.

Claim 90, as amended, is directed to a natural human erythropoietin (EPO) joined at its

carboxy-terminus by a peptide linker to the amino terminus of a human immunoglobulin domain (specific to the full-length IgG-Fc, Ch or CL domain) that does not contain a variable region, wherein the peptide linker consists of between 2 and 7 amino acid residues, wherein the amino acid residues are selected from the group consisting of: glycine and serine, and wherein said fusion protein has an EC_{50} within 4 fold of the EC_{50} of non-fused EPO, on a molar basis, in an EPO-dependent *in vitro* bioassay using a human UT7/epo cell line that proliferates in response to EPO. This combination of features is neither taught nor suggested by the combination of Blumberg, et al., and Mapelli, et al.

In view of the foregoing remarks, withdrawal of the rejection of Claims 90-94, 96, 102, 104, 105, 130-132 and 139 are rejected under 35 U.S.C. § 103(a), is respectfully requested.

Rejection Under 35 U.S.C. § 103- Blumberg, et al. in view of Mapelli, et al in further view of Qiu, et al.:

The Examiner has rejected Claims 133-135 under 35 U.S.C. § 103 (a), on the basis that the claims are unpatentable over Blumberg, et al., in view of Mapelli, et al. and further in view of Qiu et al. The Examiner refers to the arguments regarding Blumberg, et al., and Mapelli, et al. discussed above, and acknowledges that neither Blumberg, et al., or Mapelli, et al. teach a peptide linker that consists of 7 amino acids and is composed of serine and/or glycine. The rejection asserts that Qiu et al. teaches the use of a sequence comprising 3 to 7 glycine residues for the construction of dimeric EPO. Therefore, the rejection reasons that it would be obvious to combine the teachings of Blumberg, et al., Mapelli, et al. and Qiu, et al. because Qiu, et al. teaches that the use of 3-7 amino acid linkers can confer functional confirmation to the EPO dimer, and that the combined teachings provide a reasonable expectation of success of linking the components without affecting the structure and biological activity of the fusion.

As noted above, Pat. No. 6,485,726 is not an effective prior art reference, and the rejection should be withdrawn on this basis. Even if, *arguendo*, Pat. No. 6,485,726 was an effective reference, Applicant contends that Blumberg is, et al., does not actually teach or

suggest an EPO-IgG fusion protein as claimed in Claim 133-135, as asserted by the Examiner, as explained in detail below.

The rejection of Claims 133-135 under 35 U.S.C. § 103 is respectfully traversed. Qiu, et al., does not remedy the deficiencies of the teachings of Blumberg, et al. or Mapelli, et al (described above). Applicant maintains that Qiu, et al., reported that EPO-EPO fusion proteins joined by peptide linkers of 3-7 glycine residues have significantly reduced biological activities (4-10-fold) relative to wild-type EPO. Thus, Qiu, et al., does not teach that linking the components will not affect the structure and biological activity of the fusion, as asserted by the Office action.

The Applicant contends that Qiu, et al. makes conflicting statements concerning the bioactivity of their EPO dimer proteins and therefore does not teach the use of a sequence encoding 3 to 7 glycine residues for the construction of a biologically active dimeric EPO as stated by the Examiner. Qui, et al. does state that Epo dimer proteins had similar activities as Epo (page 1174, column 2, paragraph just below Fig. 2). This was based on the use of a radioimmunoassay (RIA) to measure protein Epo concentration in the conditioned media. But later on page 11175, column 1, (in the section: "Immunoblot of the engineered tag for confirmation of protein activity") Qiu, et al. states that the protein concentrations determined by RIA are incorrect and an underestimate of the amount of protein in the Epo dimer conditioned media. Therefore, the Applicant asserts that the bioactivity numbers Qiu, et al. reported in the earlier part of the reference are incorrect. In the Discussion section of Qiu, et al., page 11176, first full paragraph, Qiu, et al. states: " Interestingly, the wild type monomeric Epo is significantly more active than the wild-type dimer". . Initially, when Qui, et al. reports activities on page 11174, the activities of the proteins are expressed in mIU/mL of conditioned media (they do not purify the proteins so they are not able get an activity/mg of protein). Wild-type Epo gave 105 mIU/mL and dimeric Epo gave 185 mIU/mL. Since the activity is expressed per mL of conditioned media, it is known to those skilled in the art that it is critical to know how much of the Epo protein there is per mL of conditioned media. Initially Qiu, et al. did this by RIA and

got incorrect numbers. Later, on page 11175 Qiu, et al. got better quantitation by doing Western blots (see Fig.5 of Qiu, et al.). On page 11175 column 2 line 4 of text Qiu, et al. admits that the RIA underestimates the concentration of Epo dimers by a factor of 2-5. The Applicant contends that Qui, et al. found that an Epo dimer protein, where the two Epo proteins are separated by a small glycine linker had reduced biological activity compared to monomeric Epo. The fusion proteins as claimed in amended Claims 90 and 139 of the present invention have the EC_{50} value as Epo on an Epo molar basis. The EC_{50} of the present invention refers to activity per a specific quantity of protein, not a specific quantity of conditioned media as described by Qiu et al.

In view of the foregoing remarks, the Applicant asserts there is no motivation or expectation of success to use the combined teachings of Blumberg, et al. with Mapelli, et al. and further with Qiu, et al. to produce the fusion protein as claimed. Withdrawal of the rejection of Claims 133-135 under 35 U.S.C. § 103 is respectfully requested.

Closing Remarks

Applicant believes that the pending claims are in condition for allowance. If it would be helpful to obtain favorable consideration of this case, the Examiner is encouraged to call and discuss this case with the undersigned.

This constitutes a request for any needed extension of time and an authorization to charge all fees therefore to deposit account No. 19-1970, if not otherwise specifically requested. The undersigned hereby authorizes the charge of any fees created by the filing of this document or any deficiency of fees submitted herewith to be charged to deposit account No. 19-1970.

Respectfully submitted,

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